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Origins and taxonomic status of *Hemidactylus* geckos on the Îles Éparses of the Western Indian Ocean

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Abstract

Distinguishing between anthropogenic introductions and natural colonizations can be complex, especially in groups that are evidently both capable natural colonizers and anthropophilic, such as some geckos of the genus *Hemidactylus*. However, such information is fundamental both for constructing appropriate conservation approaches, as well as to identify biogeographical patterns. Here we assessed the origins and taxonomic status of the *Hemidactylus* geckos found on the Îles Éparses, disjunct small islands of the western Indian Ocean located around Madagascar, using partial mitochondrial DNA sequences. *Hemidactylus platycephalus* was confirmed on Juan de Nova, presumed introduced from continental Africa. The *H. mabouia-mercatorius* complex was identified on Juan de Nova, Europa, Tromelin, and Grande Glorieuse, with *H. mabouia* (*sensu stricto*) on Juan de Nova and *H. mercatorius* on Europa, from where it is likely to have been introduced to Tromelin. An unnamed lineage within *H. mercatorius*, previously known only from the Aldabra Islands, was identified on Grande Glorieuse, and further demonstrated the unexpectedly high genetic diversity within this lineage.

Key Words

16S rRNA, colonization, Europa, Glorioso, *Hemidactylus mabouia*, *Hemidactylus mercatorius*, introduction, Juan de Nova, Tromelin

Introduction

Extensive evidence indicates that invasive alien species are significant drivers of population declines and species extinctions in island ecosystems worldwide (Reaser et al. 2007). However, determining the alien status of many species is complex, since natural long-distance colonizations have been demonstrated for many different taxa. Geckos are an evident example of this, with numerous overwater dispersals across both ancient and recent timeframes indicating both a natural ability for island colonization as well as extensive, well documented anthropogenic introductions (Agarwal et al. 2021). Species of the genus *Hemidactylus* are an exemplar of this, with seven species found across the remote islands of the Indian Ocean (Rocha et al. 2022). Since neither the volcanic Mascarene islands nor the atolls of the western Indian Ocean have been connected to continental source populations, either natural or human-mediated transmarine colonizations must be invoked to explain their distribution. Separating these two phenomenona is further complicated by the convoluted taxonomic history of some of these species. In particular *Hemidactylus platycephalus* and the *Hemidactylus mabouia-mercatorius* complex were historically amalgamated as "*H. mabouia*" due to overall similar morphological aspects, so that early historical records cannot be reliably used to infer ex-

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act species presences (reviewed in Rocha et al. 2022). Disentangling these species, primarily through the use of genetic sequences, has helped elucidate colonization patterns across the Indian Ocean islands (Rocha et al. 2010a). However, most studies have still focused on larger islands, such as those of the Comoros group (Rocha et al. 2005), Madagascar (Vences et al. 2004) and Réunion (Sanchez et al. 2012). Assessment of genetic lineages, as well as confirmation of species level taxonomy, on the smaller islands are often still lacking, although these may be crucial to disentangle natural from anthropogenic introductions, and thus the native or alien status of these taxa.

Genetic data has also only partially resolved the taxonomic situation within the *Hemidactylus mabouia-mercatorius* complex. Vences et al. (2004) identified two highly divergent lineages of *H. mercatorius* within Madagascar. Rocha et al. (2005) demonstrated that individuals of *H. mabouia* from the Gulf of Guinea islands clearly grouped within their *H. mercatorius* group from northern Madagascar and Comoros. Boumans et al. (2007) identified three lineages within Madagascar, all of which were considered *H. mercatorius*. Similarly, Rocha et al. (2010a) proposed that the insular populations, including Comoros, Madagascar and the Seychelles, be treated as *H. mercatorius*, and identified three divergent groups within a paraphyletic *H. mabouia*. This would indicate that *H. mercatorius* was then also introduced to other regions, such as the Gulf of Guinea islands. More recently, comparing the data from this study with that from the comprehensive study of Agarwal et al. (2021), which found *H. mabouia* to harbor more than 20 (still unnamed) species-level lineages within which *H. mercatorius* is also embedded, led Pinho et al. (2023) to consider that one of the three groups (Clade A, group I) of Rocha et al. (2010a) actually corresponds to *H. mabouia sensu stricto*, a widespread clade both in West Africa and in the Americas, with a distribution clearly driven by anthropogenic movements. It also includes populations found in Mayotte, Mahé (Seychelles), Réunion, Zanzibar and Pemba. This means that both *H. mabouia* (*sensu stricto*) and *H. mercatorius* are found on various Indian Ocean islands, and that there have been multiple introductions in some areas, highlighting the need to assess the smaller islands.

In this study we include specimens of *Hemidactylus* from the "Îles Éparses", disjunct small islands off the western Indian Ocean, including Europa, Juan de Nova, Tromelin and Grande Glorieuse of the Glorioso islands. We sequenced part of the 16S rRNA mitochondrial gene and compared this to previously published exemplars from the larger islands to infer colonization patterns and the possible native status of these geckos on the individual islands.

Methods

The sampled islands form part of the 5th district of the French Southern and Antarctic lands, a French overseas territory. They include the main Glorioso island (Grande Glorieuse), Juan de Nova, Europa and Tromelin, with fieldwork carried out between 2013 and 2017 (Fig. 1 and Suppl. material 1). *Hemidactylus* geckos were identified in the field, and a small piece of the tail-tip removed and stored in 96% ethanol for genetic analyses. We extracted DNA from these tissues using standard High Salt methods (Sambrook et al. 1989), and then performed a PCR to amplify part of the 16s rRNA gene, using the 16SH and 16SL primers from Palumbi (1996) and the conditions described in Harris et al. (1998). Positive PCR products were cleaned and sequenced in one direction by a commercial company (Genewiz, Germany). The sequences were aligned in BioEdit using clustalW (Thompson et al. 2003), with representative sequences of the same species from GenBank.

Phylogenetic relationships were estimated using a Maximum Likelihood approach, employing PhyML 3.0 (Guindon et al. 2010), both for identifying the most appropriate model of evolution under the AIC criteria (Lefort et al. 2017), and for estimating the phylogeny. Support for the phylogeny was inferred with 1,000 bootstrap replicates. The most appropriate model of evolution identified using PhyML and therefore employed in the analysis was the GTR+I+G model for both datasets. Trees were imported to FigTree v1.4.4 for visualization.

Results

Twenty-one partial 16S rRNA sequences were generated for this study (Accession Numbers PP495142–PP495143, PP495257 and PP495518–PP495523) belonging to the *H. mabouia-mercatorius* complex (19 specimens) and *H. platycephalus* (2 specimens). Each species was analysed in a separate phylogenetic analysis, owing to differences in lengths of comparative sequences from GenBank. For *H. platycephalus* the alignment consisted of 23 sequences (494 bp), including two specimens of *Hemidactylus principensis* which were designated as outgroups. For the *H. mabouia-mercatorius* complex the alignment consisted of 120 terminals (423 bp), including divergent specimens of "*H. mabouia*" from continental Africa used to root the tree following Rocha et al. (2010a). Since all the specimens from Europa Island (n = 15) shared the same haplotype, a single representative individual was included in the analysis.

The two specimens of *H. platycephalus* sequenced from Juan de Nova shared a single haplotype, which differed by one nucleotide from a sample of this species from Mozambique (AY517572, Vences et al. 2004). These formed part of one of the three lineages previously identified by Rocha et al. (2010a), including other samples from the Comoros islands, Zanzibar and Madagascar (Fig. 2, group C). The other two lineages corresponded to clades so far restricted to Zanzibar and Pemba (Fig. 2, group A), and continental East Africa (Fig. 2, group B).

The *H. mabouia-mercatorius* complex was identified on Grande Glorieuse, Juan de Nova, Europa and Tromelin (Fig. 3). The specimen from Juan de Nova was a new

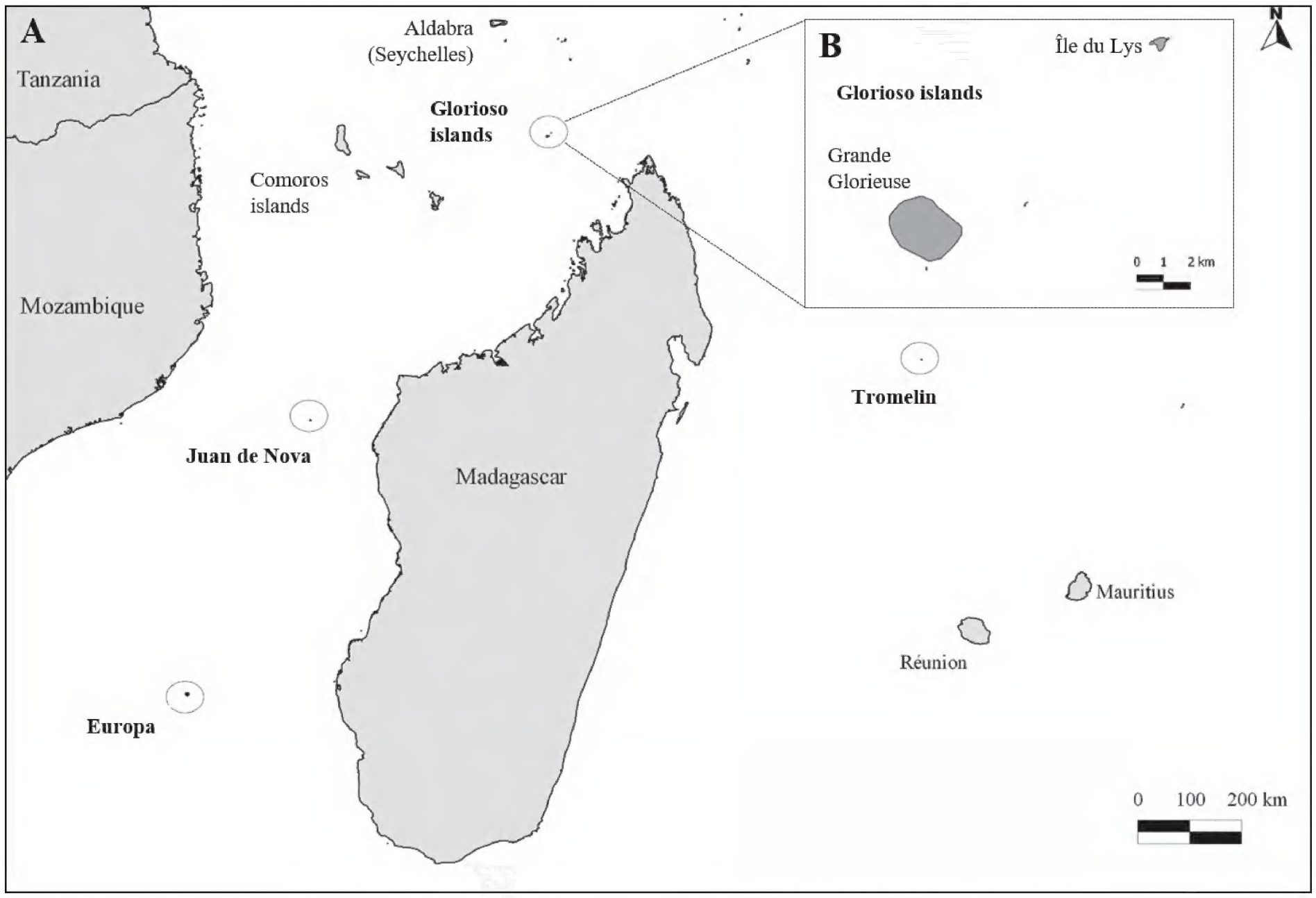

Figure 1. (**A**) The location of the Îles Éparses in the western Indian Ocean and (**B**) details of Glorioso islands.

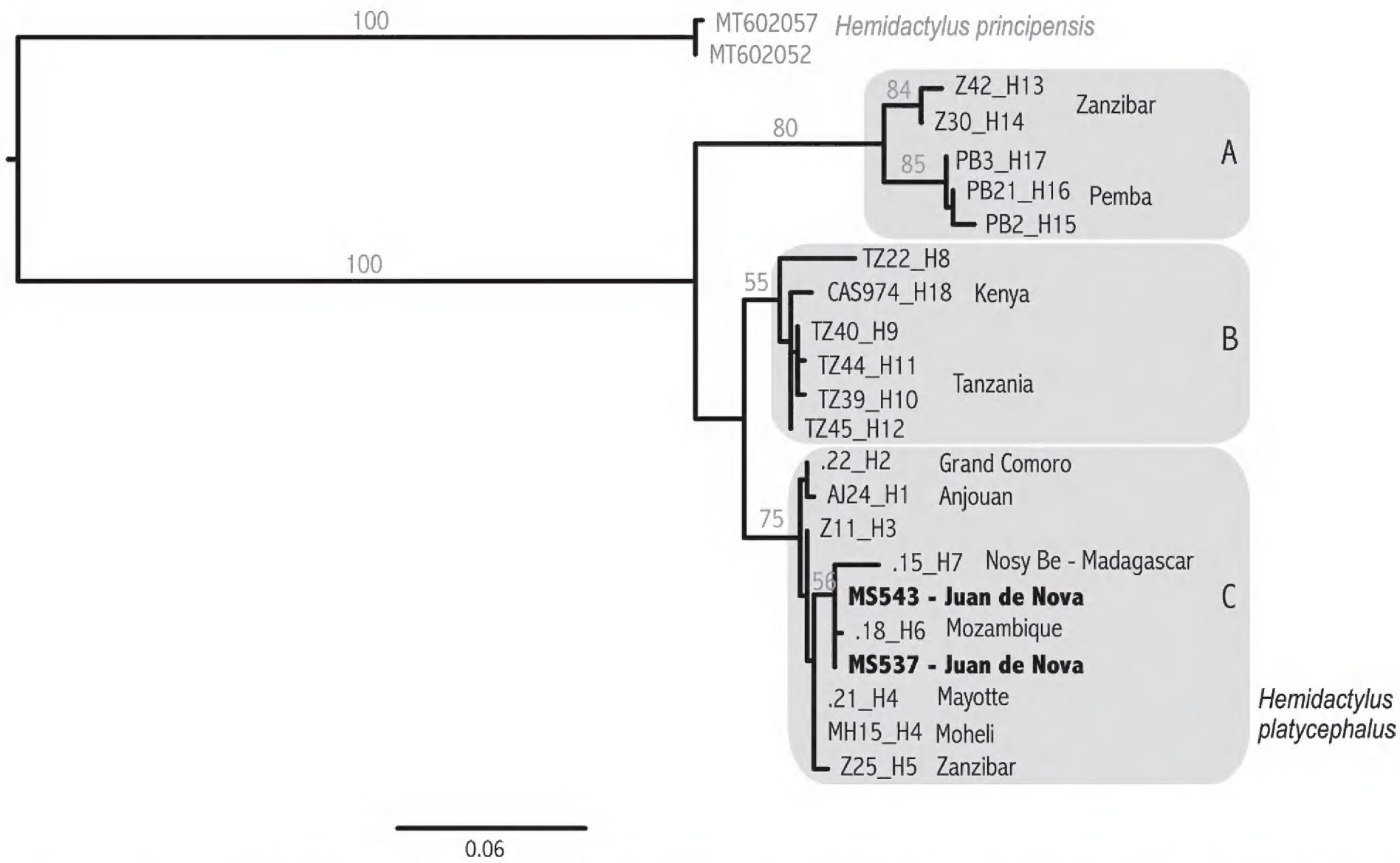

Figure 2. Estimate of relationships between populations of *Hemidactylus platycephalus* based on partial 16S rRNA sequences derived from a maximum likelihood analysis. Numbers on branches indicate bootstrap support (above 50%). Individual labeling follows Rocha et al. (2010a) for previously collected samples (sample_haplotype). Sample codes reflect geographic locations, with full details including GenBank numbers in Suppl. material 1.

haplotype, related to haplotypes previously recovered from continental Africa and the Comoros islands, as well as Réunion (Group I in Rocha et al. 2010a). Following Agarwal et al. (2021) and Pinho et al. (2023) this lineage represents *H. mabouia sensu stricto*. Specimens from Europa and Tromelin (15 and 1 respectively) shared a new haplotype, related to specimens from Madagascar (Group II in Rocha et al. 2010a, and therefore *H. mercatorius*). Specimens from Grande Glorieuse also all shared a new haplotype, embedded within a diverse clade of *H. mer-*

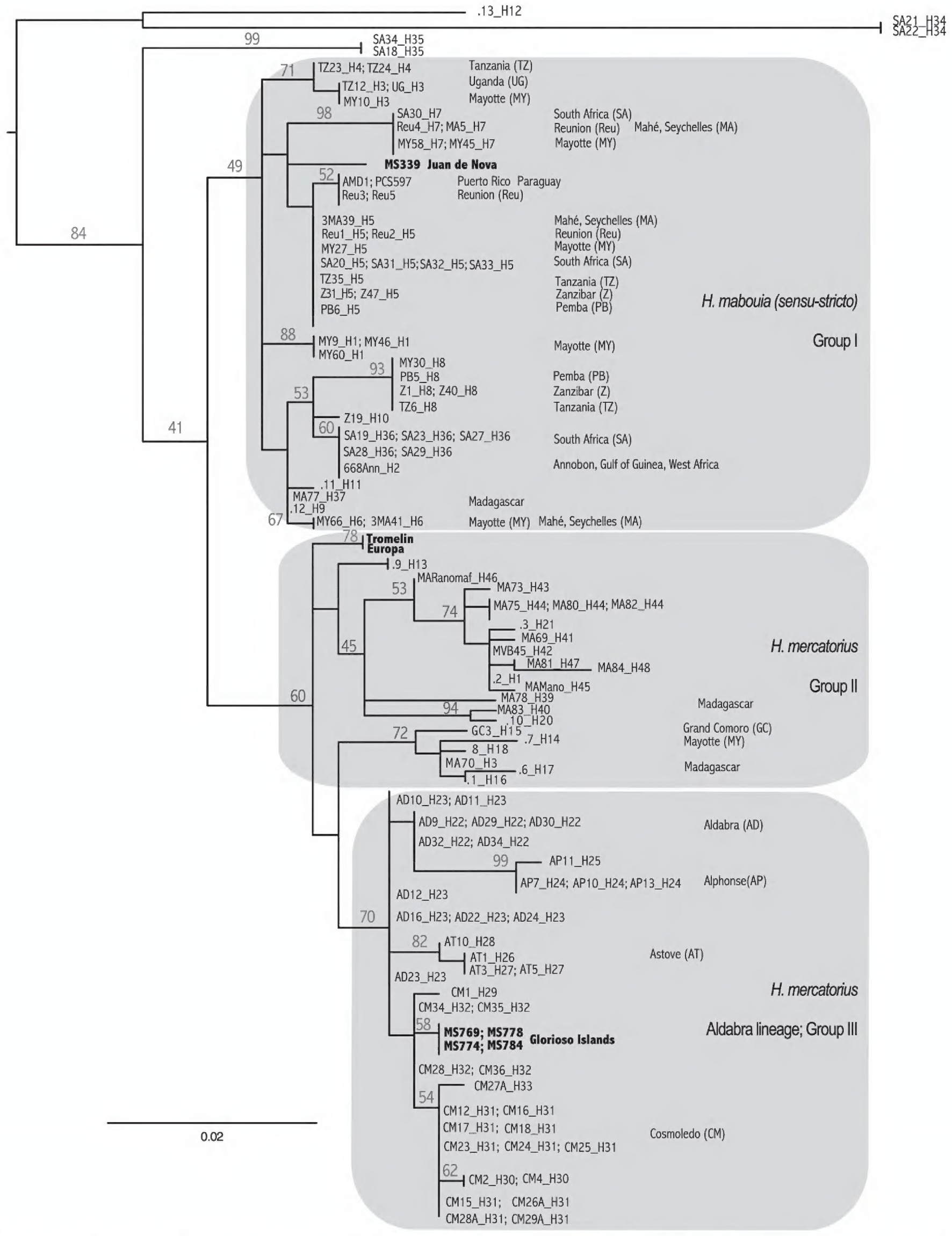

Figure 3. Estimate of relationships within the *H. mabouia-mercatorius* complex based on partial 16S rRNA sequences derived from a maximum likelihood analysis. Numbers on branches indicate bootstrap support (above 50%). Groups I–III (and individuals) are labelled following Rocha et al. (2010a) for previously collected samples (sample_haplotype). Sample codes reflect geographic locations, with full details including GenBank numbers in Suppl. material 1.

catorius, previously identified only from the coralline archipelago of Aldabra, including Aldabra, Cosmoledo, Astove and Assumption (Group III in Rocha et al. 2010a).

Discussion

Distinguishing native from alien species is a first step towards answering many biogeographic and evolutionary questions, and for addressing key conservation concerns. Attempts to define approaches to identify alien species usually propose various criteria, while recognizing that these are generally indirect evidence and that rather than recognizing alien status based on a single criterion, only the existence of several criteria together can result in a high classification probability (e.g. Orlova-Bienkowskaja 2016). Low genetic diversity, high frequency of known naturalization, unexpected geographic distribution and lack of historical records are all criteria often proposed as indicators for alien status (e.g. Webb 1985).

In the case of *Hemidactylus* geckos in Indian Ocean islands, most of these criteria are not particularly informative. Frequency of naturalization is high for some species, particularly the *H. mabouia* complex, which has "conquered the world", although this contains up to 20 putative species, only one of which is invasive and widely distributed (Agarwal et al. 2021). Similarly, *Hemidactylus* species have undoubtedly reached many islands naturally, so that the geographic occurrence on even relatively young islands cannot be used to discount native status. House geckos tend to be recorded less than more conspicuous mammals and birds, so early historical records are mostly limited to a few reports by naturalists, and even then the notable morphological similarity between many species means that species-level identifications can be suspect if museum specimens were not collected. On the other hand, early records on some isolated islands have been considered to suggest the possibility of early (pre-European) or natural dispersals for some species (reviewed in Rocha et al. 2022). Regarding the Îles Éparses, records indicate "*H. mabouia*" on Glorioso and Juan de Nova since the 1890's, and on Europa since 1903 (reviewed in Sanchez et al. 2019), which while relatively early historically still leave open the possibility of anthropogenic introductions. *Hemidactylus platycephalus* was only identified with certainty on Juan de Nova and Glorioso in the last 25 years, as was *H. mercatorius* on Tromelin (reviewed in Sanchez et al. 2019). This leaves genetic data as one of the few options left to identify potential status on most islands.

Mitochondrial DNA sequences have, in some cases, given compelling additional evidence regarding the status of some populations of *Hemidactylus* on Indian Ocean islands. In the case of the population of *H. mabouia* (initially considered as *H. mercatorius*) from Réunion, three haplotypes were identified from a partial 16S rRNA fragment, two of which were immediately reported as having been found in populations from Africa and the Comoros (Sanchez et al. 2012), while the third (individuals Reu 3 and Reu 5) is identical to specimens since collected in Puerto Rico (GenBank accession KC840516, Diaz-Lameiro et al. 2013). These all belong to the lineage now considered as *H. mabouia sensu-stricto* (probably clade "b", although there is low resolution in the 16S rRNA sequence data to recover the two clades identified by Agarwal et al. (2021) using ND2 sequence data), which is associated with anthropogenic movements and widespread across Africa and the Americas. In this case, the genetic data therefore strongly supports recent, anthropogenic introduction(s). Furthermore, the *H. mabouia-mercatorius* complex (group I of Rocha et al. 2010a) is now clearly identified as *H. mabouia sensu stricto* (Pinho et al. 2023), implying that these populations found in Mayotte (Comoros), Mahé (Seychelles), Zanzibar and Pemba (Tanzania) and some primarily coastal localities of Tanzania and South Africa are highly likely introduced through human-aided transport, which seems to be the predominant pattern of this species. Regarding the Îles Éparses, the finding of the same unique haplotype in specimens from Europa and Tromelin, along with the lack of older records from Tromelin and the large distance between these two islands, also indicates a recent introduction of *H. mercatorius* to Tromelin from Europa – currently logistic support boats land on each island consecutively every two to three years to remove waste material, and this seems a plausible colonization pathway. Likewise, the lack of historical records of *H. platycephalus* on Juan de Nova and the high genetic similarity with specimens from continental Africa indicates a recent introduction. However, the situation of the populations of *H. mercatorius* on Europa and *H. mabouia* on Juan de Nova is less clear-cut, since both islands accommodate specimens with distinct haplotypes, quite different from any currently available for comparisons (Fig. 3). Perhaps this fact is due to a lack of sampling across likely source populations. A similar situation occurs with *Hemidactylus* species in the Maldives, where attempts to illuminate colonization patterns are hampered by incomplete information on the natural variation from continental source populations (Agarwal et al. 2019). Interestingly, Europa seems to have been colonized with *H. mercatorius* from Madagascar, while Juan de Nova with *H. mabouia* from continental Africa, and this same pattern occurs in *Lygodactylus* geckos, with *Lygodactylus verticillatus* from Europa conspecific with Malagasy populations, while *Lygodactylus insularis* from Juan de Nova is embedded within the *Lygodactylus capensis* group, with an African origin (Röll et al. 2023). On the other hand, the skink *Trachylepis maculilabris* from Europa appear closely related to populations from Mozambique (Rocha et al. 2010b), meaning that no clear biogeographical pattern is evident from the reptiles of this island. Further, without more data from potential source populations, the native or introduced status for *Hemidactylus* from these islands remains debatable.

The identification of a new haplotype within *H. mercatorius* from Grande Glorieuse, embedded within the "Aldabra clade" (*sensu* Rocha et al. 2010a), is of particular interest. It could be interpreted as a recent introduction from Aldabra, given that concessions to exploit coconut plantations on the islands were given to Seychelles companies during the 20th Century. On the other hand, a high diversity on Aldabra is quite unexpected given the relatively recent complete inundation of this atoll (around 136,000 years ago, reviewed in Hume et al. 2018). Previous haplotype diversity identified by Rocha et al. (2010a) was already high (12 in total, across the four islands of the Aldabra archipelago sampled), and the finding of another unique haplotype on Grande Glorieuse increases the known diversity within this clade. Many authors of phylogeographic assessments in this region suggest that compared to Aldabra, the other islands of the group are much younger, around only 15,000 years (e.g. Radkey 1996; Warren et al. 2003). However, this evidence seems to be based on geological assessments focused on Aldabra (e.g. Taylor et al. 1979). Korotky et al. (1990) identified multiple marine terraces on Assumption, some of which are higher than those on Aldabra, suggesting these islands have similar ages (Boyden et al. 2021). Having multiple, older islands in relative proximity – Assumption Island is only 27 km from Aldabra – may help explain the diversity identified in this clade, endemic to the archipelago, and might also explain the appearance and extinction of two or even three other gecko species on Aldabra which are present in the fossil record after the inundation about 100,000 years ago (Arnold 1976). While their extinction may have been associated with the formation of the central lagoon on Aldabra around 5000 years ago (reviewed in Hume et al. 2018), their presence after the inundation indicates that Aldabra was being regularly colonized by reptiles over an evolutionary short time period. Overall, given these alternative scenarios, examination of other flora and fauna within the Aldabra archipelago for similar genetic signatures of longer persistence in the area should be performed.

To conclude, on the Îles Éparses, while some signals of recent anthropogenic introductions were identified (*H. platycephalus* on Juan de Nova and *H. mercatorius* on Tromelin), the status of the populations of *H. mabouia* on Juan de Nova and *H. mercatorius* on Europa remains ambiguous. On the other hand, *H. mercatorius* from Grande Glorieuse is part of a clade previously identified on the Aldabra archipelago, adding to this unexpectedly diverse endemic insular lineage. Further sampling in Madagascar and continental Africa, as well as assessment of more taxa from Aldabra, will be needed to further address these complex biogeographical questions.

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Supplementary material 1

Supplementary DNA sequence data

Authors: D. James Harris, Mickaël Sanchez, Sara Rocha
Data type: docx
Explanation note: The supplementary material includes a full list of all samples included in the analyses, with GenBank accession numbers, sample codes and geographic localities.
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